REMARKS

The present invention relates to regulatory T cells (Treg cells) and methods of long-term, culture-expanding, activating and using same in immunotherapy and for the suppression of autoimmune responses.

By way of the present Amendment, claim 1 has been amended to indicate that the isolated population of human $CD4^+CD25^+$ Treg cells are further culture-expanded in the presence of a $CD4^+$ feeder cell or $CD4^+$ feeder cell conditioned medium, thereby producing therapeutic human Treg cells with enhanced suppressor activity, wherein said culture expanded Treg cells are $CD62L^+/CD27^+$ and capable of inhibiting proliferation of $CD4^+CD25^-$ responding T cells in a MLR assay by at least 90%. Support for this amendment is found through out the specification (e.g., See Example 8) as more fully discussed elsewhere herein. No new matter has been added by way of these amendments.

Claims 4 and 33 have been amended to have proper antecedent basis. No new matter has been added.

Response to Rejections Under 35 U.S.C. 112, Second Paragraph

Claims 2, 3, and 32 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the phrases "comprises a high level of stringency" and "wherein isolation step further comprises substantially enhancing $CD4^+CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population". While not necessarily agreeing with the Examiner, in an effort to advance prosecution of the claims, Applicants have canceled claims 2, 3, and 32, thereby rendering this rejection moot.

Rejection of claims 1-5, 7-11, and 28-36 under 35 USC §112, 1st paragraph - enablement

The Examiner has rejected claims 1-5, 7-11, and 28-36 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement standard. The Examiner contends that the specification does not reasonably provide enablement for human Tregs with *enhanced suppressor activity*. Applicants respectfully submit that the claimed invention is enabled by the specification as filed under the current law pursuant to 35 U.S.C. § 112, first paragraph, for the following reasons.

As an initial matter, Applicant enjoys a presumption that the specification, which discloses how to make and use the claimed invention, complies with the first paragraph of 35 U.S.C. §112, unless there is a reason to doubt the objective truth of the specification. See, *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971). The initial burden of establishing a basis for denying patentability to a claimed invention rests upon the examiner. See, *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Thorpe*, 777 F.2d 695, 227 USPQ 964 (Fed. Cir. 1985); *In re Piasecki*, 745 F.2d 1468, 223 USPQ 785 (Fed. Cir. 1984).

It is well-settled that an Applicant need not have actually reduced the invention to practice prior to filing. MPEP §2164.02 (citing *Gould v. Quigg*, 822 F.2d 1074 (Fed. Cir. 1987)). Indeed, the invention need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908 (C.C.P.A. 1970). The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991)). Therefore, under current law, enablement does not require a working example and experimentation is allowed so long as it is not undue. The present case law regarding enablement under 35 U.S.C. §112, first paragraph, allows significant experimentation without finding it undue if the art typically engages in such experimentation.

Applicants submit that the present Experimental Examples, taken in view of the specification, provide abundant guidance to the skilled artisan to practice the claimed invention. In addition, claim 1 has been amended herein to indicate that the Treg cells with enhanced suppressor activity are $CD62L^{+}/CD27^{+}$ and capable of inhibiting proliferation of CD4+CD25- responding T cells in a Mixed Lymphocyte Reaction (MLR) assay by at least 90%. Support for this amendment is found through out the specification for example in Example 8. Thus no new matter has been added.

Example 8 provides evidence that human Tregs with *enhanced suppressor activity* were successfully generated using the claimed method. Example 8 demonstrates that suppressor

cells can be isolated and culture expanded from human blood. In some instances, the cultured Tregs cells can be expanded over 100-fold, and when relatively pure, exhibit enhanced and potent suppressive activity compared to a population of cells not generated using the methods of the invention. The suppressor function of the cells of the invention in MLR assays was shown to almost completely block HLA mismatched MLR in Example 8.

In an effort to determine the differences between the weakly and potently suppressive cell lines, the inventors analyzed cell surface antigen between the cell populations and observed that CD62L and CD27 were expressed on a higher percentage of cells in the potent suppressor cell lines compared with weak lines (Figures 18G, 18H, 18I). The inventors also evaluated the functional relevance of expression of these markers. CD62L+ or CD27+ cells were isolated and found enrichment for suppressor activity in both of the positive subsets. In addition, the inventors in an effort to more definitively determine function of these cell line subsets, sorted the cells for CD62L+/CD27+, CD62L+/CD27-, and CD62L-/CD27+ cells (Figure 18J) and tested each population of cells for suppressor activity in an MLR assay. It was observed that suppressor function was solely within the CD62L+/CD27+ subset.

With respect to the suppressor function, the cells were characterized in the context of MLR assays. It was observed that the cell lines were able to suppress respondent cells by >90% as measured on day six of the MLR assay. To quantify the minimum number of suppressor cells required for potent inhibition, titrated numbers of suppressor cells were added to MLR cultures. The titration curves (Figure. 19) revealed an approximate break point at a suppressor-to-responder ratio of less than 1:10 (5,000 suppressors to 50,000 responders).

The claims are enabled and for the reasons given, Applicants respectfully request reconsideration and withdrawal of the rejection pursuant to 35 U.S.C. §112, first paragraph.

Rejection of claims 1-5, 7-11, and 28-36 under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-5, 7-11, and 28-36 under 35 U.S.C. § 103(a) as being unpatentable by Schuler *et al.* (US2005/0101012) in view of Baecher-Allan et al. and CD25 Microbead Datasheet (as evidenced by Elkord, 1996 Biocompare Review). Specifically, the Examiner contends that Schuler teaches contacting a sample of $CD4^+$ T cells with anti-CD25 antibody to produce an isolated population of human $CD4^+CD25^+$ T cells and expanding the $CD4^+CD25^+$ T cells with anti-CD3 and anti-CD28 antibodies. Therefore, the Examiner contends

that it would have been obvious to one of skill in the art to use the teachings of Schuler with Baecher-Allan to adjust the cell/bead ratio to isolate $CD4^+CD25^+$ T cells and to expand the isolated cells using anti-CD3 and anti-CD28 antibodies. Applicants respectfully traverse this rejection, and respectfully submit that the combination of art cited by the Examiner does not render the claims obvious under 35 U.S.C. § 103(a) for the following reasons.

According to the U.S. Supreme Court ruling in *Graham v. John Deere*, 383 U.S. 1 (1960), in making a case for obviousness, the Examiner must 1) determine the scope and content of the prior art; 2) ascertain the differences between the prior art and the claims at issue; 3) resolve the level of ordinary skill in the pertinent art; and 4) evaluate evidence of secondary considerations. These principles have been reconfirmed by the Supreme Court in *KSR International Co. v. Teleflex Inc.*, 550 USPQ2d 1385 (2007).

In *KSR Int'l Co.*, the US Supreme Court restated the requirements for a finding of obviousness. Encouraging the application of common knowledge and common sense, the Court took care to guard against hindsight bias and *ex post* reasoning and to distinguish the predictable from the unpredictable arts ("If a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability." [Emphasis added]). Based on the combination of references set forth by the Examiner, Applicant asserts that the rejection of the claims under §103 could only have been made with hindsight bias and *ex post* reasoning.

When applying 35 U.S.C. § 103, the following tenets of patent law must be followed: 1) the claimed invention must be considered as a whole; 2) the references must be considered as a whole; 3) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and 4) reasonable expectation of success is the standard with which obviousness is determined (MPEP § 2141 II). None of these criteria have been met here.

With respect to Schuler, Applicants contend that Schuler alone or in combination with the other references does not render the present claims obvious. While Schuler reported 95% purity of CD4+CD25+ cells, Schuler was not able to induce proliferation of the cells and have the cells exhibit suppressor function. In paragraph [0083], Schuler states the "[t]he exceedingly low proliferative response of CD4+CD25+ T cells was also apparent when these cell populations were polyclonally stimulated with platebound anti-CD3 soluble anti-CD28". Moreover, Schuler in [0088] indicates that promotion of proliferation of CD4+CD25+ T cells

reduced their inhibitory effects. Thus, the disclosure by Schuler indicates that Schuler was not successful in inducing proliferation of CD4+CD25+ T cells, and when a low level of proliferation did occur, the CD4+CD25+ T cells lost their inhibitory activity. This teaches away from the present invention which relates to culture expanding CD4+CD25+ T cells where the cells maintain their suppressor function. The present invention fulfills the need in the art for methods of producing sufficient number of Treg cells to permit characterization and to provide for safe and effective therapeutic use in human patients.

The present invention is based on the discovery that Tregs can be expanded using the methods of the invention, whereby following expansion, the cells exhibit potent suppressor activity. The inventors observed that contamination of $CD25^{dim}$ cells in $CD25^{+}$ fractions grew faster and overgrew the $CD25^{bright}$ cells, and thereby precluded the full manifestation of suppressor cell function (*See, e.g.*, Example 8). It was observed that $CD25^{dim}$ cells exhibited a lower suppressive activity than $CD25^{bright}$ cells (*See, e.g.*, paragraph 24, page 8). Schuler did not even recognize as the inventors recognized that it is advantageous to at least isolate the $CD25^{bright}$ subset of $CD4^{+}CD25^{+}$ cells in order to detect suppressor activity.

The result of using the double column purification methodology of the invention is the generation of $CD4^{+}CD25^{+}$ cells that exhibit potent functional suppressor activity following cell expansion (*e.g.*, >90% inhibition). When assayed, the culture-expanded human suppressor cells of the present invention were capable of about >90% suppression of an MLR, either with fresh $CD4^{+}$ cells or cultured $CD4^{+}CD25^{-}$ cells as responding T cells (*See, e.g.*, Example 8). The inventors recognized that a very high level of stringency was critical for the isolation of human cells of sufficient purity for suppressor cell line generation. The methods of Schuler are inadequate for the isolation and expansion of CD4+CD25+ cells for the generation of the suppressor cell lines or culture expanded $CD4^{+}CD25^{+}$ cells that exhibit potent functional suppressor activity.

With respect to the teachings of Baecher-Allan, Applicants submit that this reference teaches away from the present invention. One page 1248, Baecher-Allan discloses that CD4+CD25+ cells costimulated with CD28 cross-linking and anti-CD3 resulted in both CD4+CD25+ cell proliferation (albeit at a low level) and loss of regulation. This is contrary to the present invention where anti-CD3 and anti-CD28 is used to induce proliferation of

CD4+CD25+ cells for the generation of a population of CD4+CD25+ cells with enhanced suppressor activity.

Contrary to the Examiner's opinion, the results summarized in Figure 5 are not applicable to the present invention because the experiments were designed to assess the dependence of CD4+CD25+ T cell-mediated suppression on cell contact (e.g., co-culture assay). This is not the same as the Examiner's assertion that Figure 5 demonstrates culturing CD4+CD25+ cells with feeder cells or conditioned media derived from feeder cells. The results in Figure 5 demonstrate that CD4+CD25+ T cells inhibit proliferation and cytokine secretion induced by TCR cross-linking of CD4+CD25- responder T cells in a contact-dependent manner. The co-culture assay for determining the suppressor activity of CD4+CD25+ cells is not applicable to culturing CD4+CD25+ cells with feeder cells (e.g., irradiated CD4+CD25-) for the purpose of proliferating CD4+CD25+ cells (e.g., assessing suppressor function of a cell is different from inducing cell proliferation). Therefore, Figure 5 cannot be relied upon to demonstrate that Baecher-Allan discloses the use of CD4+CD25- cells as feeder cells in the context of proliferating CD4+CD25+ cells for generating a sufficient amount of CD4+CD25+ cells. Nothing in Figure 5 is directed to the use feeder cells for the purpose of providing factors for culturing CD4+CD25+ cells. In a non-limiting example, Example 8 discloses that irradiated CD4+CD25- cells were used to provide factors useful for the proliferation of CD4+CD25+ cells.

Claim 1 has been amended to indicate that the isolated cells are further cultured in the presence of a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium. Nowhere does Baecher-Allan or Schuler teach culturing CD4+CD25+ cells on a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium as encompassed in the pending claims. Accordingly, Baecher-Allan or Schuler alone or in combination with the other references can render the present claims obvious.

The inventors have developed methods for improved cell isolation and culturing. It was observed that the more stringently the $CD4^+CD25^+$ cells were purified, the less well they grew in culture, even with stimulation with anti-CD3/CD28 beads and IL-2. However, after trying various accessory cell populations, irradiated $CD4^+$ T cells (used as "feeder cells") were found to yield the best results for facilitating growth of the isolated $CD25^+$ bright cells. It was also observed that conditioned media (supernatant from anti-CD3/anti-CD28 stimulated $CD4^+$ T

cells) greatly facilitated suppressor cell growth, even more so than that which resulted from IL-2 supplementation.

Accordingly, a preferred embodiment of the present invention encompasses isolating a population of $CD4^+CD25^{bright}$ cells using a highly stringent purification system (e.g., double column purification procedure) and culture expanding the $CD4^+CD25^{bright}$ using anti-CD3/28 mAb coated beads in combination with IL-2 and/or irradiated feeder cells to induce both (i) robust expansion by >100-fold and (ii) an increase in suppressor cell activity.

Nothing in Baecher-Allan, Schuler, and CD25 Microbead Datasheet (as evidenced by Elkord, 1996 Biocompare Review) disclose culture expanding isolated Tregs in the presence of a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium wherein the Tregs maintain their suppressor function. In fact, when Baecher-Allan and Schuler attempted to induce proliferation of CD4+CD25+ cells, their cells lost inhibitory function. For the reasons discussed above, the combination of Schuler with Baecher-Allan and CD25 Microbead Datasheet does not render the claims obvious under 35 U.S.C. § 103(a) and, therefore, the rejection should be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that the claims are now in condition for allowance. Reconsideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

BRUCE BLAZAR ET AL.,

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(Date)

By: _____

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